PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [6] : A61J 1/00, A61M 1/02, G01N 21/31	A1	(11) International Publication Number: WO 98/38961
		(43) International Publication Date: 11 September 1998 (11.09.98)

<table>
<tr><td>

(21) International Application Number: PCT/CA98/00170

(22) International Filing Date: 3 March 1998 (03.03.98)

(30) Priority Data:
60/038,555   3 March 1997 (03.03.97)   US

(71) Applicant *(for all designated States except US)*: CME TELEMETRIX INC. [CA/CA]; 560 Parkside Drive, Waterloo, Ontario N2L 5Z4 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SAMSOONDAR, James [CA/CA]; 5556 Whistler Crescent, Mississauga, Ontario L4Z 3R5 (CA). GIVEN, Douglas, George [CA/CA]; 474 Timbercroft Crescent, Waterloo, Ontario N2T 2J2 (CA).

(74) Agent: RUDOLPH, John, R.; Gowling, Strathy & Henderson, Suite 1020, 50 Queen Street North, Kitchener, Ontario N2H 6M2 (CA).

</td><td>

(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

BEST AVAILABLE COPY

</td></tr>
</table>

(54) Title: METHOD AND APPARATUS FOR SCREENING PLASMA FOR INTERFERENTS IN PLASMA FROM DONOR BLOOD BAGS

(57) Abstract

An apparatus and a method whereby plasma integrity of plasma contained in a blood bag is rapidly and accurately assessed without compromising the sterility of the plasma, or destroying any of its components. This is achieved through spectral data which is used in a novel way so as to determine if a plasma specimen representative of plasma in a blood bag contains interferents and if so, to what extent. The apparatus and method analyse plasma contained in two bags whereby tubing connects the two bags. A lamp is used to irradiate the specimen, and a spectrophotometer is used to measure radiation from the specimen. The apparatus and method allow for determination where both the bags and tubings are translucent and contain writing on their surfaces (e.g., proprietary information), and the light is transmitted through the writings, plastic, and the plasma contained in the bag or tubing.
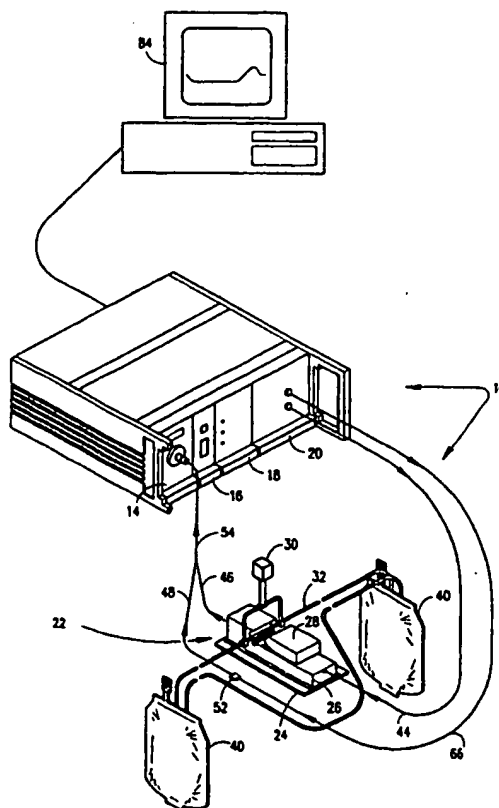
METHOD AND APPARATUS FOR SCREENING PLASMA FOR INTERFERENTS IN PLASMA FROM DONOR BLOOD BAGS

Field of Invention

This invention relates to spectrophotometry and the spectrophotometric analysis of plasma expressed from donor blood bags. In particular, this invention relates to a method and apparatus for providing a rapid non-destructive measurement of substances called interferents which compromise plasma integrity, by measurement of absorbance or reflectance. Furthermore, the spectrophotometric measurements are made after the plasma is expressed from the primary blood donor bags, without altering the sterility of the blood components.

Background of Invention

Blood is usually donated into sterile plastic bags which contain anticoagulants. These bags ("blood bags") are connected to one or more similar bags by plastic tubing in a closed system for maintaining sterility. After centrifugation of whole blood contained in a primary collection bag, plasma or plasma plus platelets can be separated from red blood cells in the bag: a higher centrifugal force can separate all cellular elements from the plasma, and a lower centrifugal force can separate the plasma plus platelets from the red cells; the plasma plus platelets can then be subjected to higher centrifugal force in order to separate the platelets from the plasma. Therefore, if separation of plasma, platelets, and red cells is required, a two-step centrifugation is necessary, with a primary blood bag linked to two "satellite" bags in series. If separation of all cellular elements from plasma is required, a single-step centrifugation is necessary, with the primary blood bag linked to one satellite bag. In both cases, plasma will be contained in the last bag having transferred to this last blood bag via plastic tubing from the other bags.

1

interferent. In fact, if a sample is sufficiently contaminated with interferents, tests are normally not conducted as the results will not be reliable.

In blood banking, plasma with compromised integrity will be discarded. Plasma specimen integrity is an essential part of quality assurance as it directly affects the accuracy of test results, and the suitability of the plasma for transfusion or fractionation. Measurement of MB provides additional assurance that the plasma contains the required amount of MB.

Spectrophotometric measurement typically employs infrared (IR) or near infrared radiation (NIR) to assess the concentration of various constituents in a blood sample. Examples of photometric measurements using containers which hold a blood sample are disclosed in U.S. Patent Nos. 5,291,884; 5,288,646; 5,066,859; and 5,366,903.

U.S. Patent No. 5,366,903 discloses a sampling device which allows photometric quantitative determination of an analyte in whole blood. The device overcomes the problems of having blood cells in a blood sample by effectively "squeezing out" red blood cells and providing a small volume of sample, free of red blood cell material, from which particular analytes can be measured.

Other applications of photometric methodology include non-invasive determinations of analyte concentrations such as described in U.S. Patent Nos. 5,360,004; 5,353,790; and 5,351,685. However none of these documents discloses a method of measuring interferents in the plasma or serum of a blood sample, in order to assess specimen integrity with respect to blood tests, plasma transfusion, or plasma fractionation.

Current methods used for detecting haemoglobinemia, bilirubinemia, biliverdinemia and lipemia or turbidity utilize visual inspection of the specimen with or without comparison to a coloured chart. It is to be understood that those practising in the field use the terms lipemia and turbidity interchangeably. This is because lipemia is the major cause of turbidity in serum or plasma. In blood banking, turbidity is assessed by the ability to read print on a paper placed behind a plasma bag.

two bags and tubing connects two bags using a lamp to irradiate the specimen, and a spectrophotometer to measure radiation from the specimen.

In still another aspect of the invention, both the bags and tubings are translucent and contain writing on their surfaces (e.g., proprietary information), and the light is transmitted through the writings, plastic, and the plasma contained in the bag or tubing.

In yet another aspect of the invention, there is provided an apparatus and a method for determining plasma integrity, where the light is reflected from a reflective surface placed behind the plasma bag or the tubing connecting the bags.

In another aspect of the invention, plasma integrity of plasma contained in a blood bag is assessed by measuring:

1.   Haemoglobin concentration as an assessment of haemolysis;

2.   Bilirubin concentration as an assessment of bilirubinemia;

3.   Biliverdin concentration as an assessment of biliverdinemia;

4.   Equivalent intralipid concentration for the assessment of turbidity; and

5.   Methylene blue concentration as part of the viral inactivation quality assurance system.

In one embodiment, Hb concentration is determined by measurement of absorption of different wavelengths of light in plasma specimens contained in a blood bag which are then compared with values obtained through calibration using reference measurements for haemoglobin in plasma specimens. Turbidity, in equivalent grams per liter Intralipid™ (IL), is determined by measurement of absorption of different wavelengths of light in the blood bag plasma specimens which are then compared with values obtained through calibration using serum samples spiked with known amounts of IL; IL is a fat emulsion in water which is similar to naturally-occurring chylomicrons, and may be used to simulate turbid serum or plasma specimens. BR concentration is determined by a combined measurement of absorption of different wavelengths of light in the blood bag plasma specimens which are then compared with values obtained through calibration

measurements for MB in plasma specimens. On the basis of the results from measurement of any one or more of these interferents at a time, in comparison with reference measurements of various levels of interferents, a decision is made concerning whether to reject or accept the plasma contained in the blood bag.

In still another embodiment of the invention, a bag of plasma is placed in a box which houses a V-shaped receptor for a corner of the bag. Light is transmitted through the plasma in the bag, and the receptor maintains a constant pathlength across the walls of the "V," for any bag.

In yet a further embodiment the present invention provides an apparatus for determining plasma integrity of plasma where the apparatus comprises: a housing, which does not have to be light-tight, for receiving a sample; a radiation source; a spectrophotometer with appropriate filters, a grating and a linear photodiode array (PDA) detector; a means for optically connecting the radiation source with the detector along a sample path through the housing and along a reference path which by-passes the sample; a means for selectively passing a beam from the sample path and from the reference path to the detector; a means for selecting an appropriate integration time required for adequate detector response; and a means for correlating a detector response, from the sample path relative to a detector response from the reference path, to a quantity of a known substance in said sample.

The apparatus further comprises a quartz-tungsten-halogen bulb capable of emitting a near infrared, and adjacent visible region light beam having wavelengths from 475nm to 1075nm and a single optical fibre bundle which randomly samples light from the quartz-tungsten-halogen bulb. The single fibre bundle bifurcates into a sample path beam for travel along a sample path and a reference path beam for travel along a reference path. The bifurcated optical fibre consists of multiple fibres which focus random sampling of light from the lamp, into single fibres of 0.4 millimeter diameter for both the sample and reference beams. This apparatus further comprises two shutters, installed in the lamp assembly, for selectively blocking the

holds tubing from a blood bag which housing is not completely light-tight: room light leakage occurs along the tubing which sticks out of the sample holder. A method of the invention provides that the light leakage is compensated for by measuring dark current, i.e., detector response when detector is not exposed to instrument light, for both sample and reference measurements. Two shutters in the apparatus are located in the lamp assembly, and are used for sequentially directing light through the sample or reference pathway. Since there is no shutter between the sample housing and the sensor, any room light leakage into the sample housing will affect sample light and sample dark scans equally when performed at the same integration time, and the reference light and reference dark scans when performed at the same integration time used for the reference measurements. Therefore, room light impinging on the detector can be effectively subtracted without affecting the performance of the apparatus, provided that the ambient light does not change during the few seconds measurement time.

In a further aspect of the present invention the apparatus provides a means for determining specimen integrity of a sample by determining the concentrations of a known substance which is selected from a group comprising haemoglobin, Intralipid, bilirubin, biliverdin, and methylene blue.

In yet a further embodiment of the present invention a method is provided for determining plasma integrity of plasma contained in a blood bag, wherein the method comprises the following steps. First, transmitting a beam of radiation along a sample path through a sample of the plasma and along a reference path by-passing the sample. Next, selectively receiving the beam of radiation from the sample path and the reference path, and analyzing the received beams of radiation from the sample path and from the reference path for an amplitude of at least one predetermined light frequency. Finally, correlating the absorbance of at least one predetermined light frequency with a quantity of a known substance.

In a further aspect of the present invention there is provided a method for determining plasma integrity of plasma contained in a blood bag, wherein

Figure 10   is a graphic representation of a linear regression fit of data for methylene blue calculation in units of micrograms per decilitre (mcg/dL) on the abscissa and ordinant axes;

Figure 11   is a graphic representation of a linear regression fit of data in respect of predicted haemoglobin concentration for samples not used in the calibration process, in units of grams per litre on the abscissa and ordinant axes;

Figure 12   is a graphic representation of a linear regression fit of data in respect of predicted intralipid concentration for samples not used in the calibration (using 988nm and 1038nm) process, in units of grams per litre on the abscissa and ordinant axes;

Figure 13   is a graphic representation of a linear regression fit of data in respect of predicted intralipid concentration for samples not used in the calibration (using 874nm) process, in units of grams per litre on the abscissa and ordinant axes;

Figure 14   is a graphic representation of a linear regression fit of data in respect of predicted biliverdin concentration for sample not used in the calibration process, in units of milligrams per decilitre on the abscissa and ordinant axes;

Figure 15   is a graphic representation of a linear regression fit of data in respect of predicted bilirubin concentration for sample not used in the calibration process, in units of milligrams per decilitre on the abscissa and ordinant axes;

Figure 16   is a graphic representation of a linear regression fit of data for predicted methylene blue concentration for samples not used in the calibration process, in units of micrograms per decilitre (mcg/dL) on the abscissa and ordinant axes.

Description of the Invention

A system incorporating the apparatus of the present invention is generally shown in Figure 1. The apparatus 10 comprises a spectrophotometer 14 optically coupled to a sample holder 22 through single

Referring to Figures 2 and 3, optical fibre 44 extends through a bore 36 in a wall of the sample holder as shown such that the end of optical fibre 44 communicates with cavity 42 to transmit radiation therein. Similarly, optical fibre 46 extends through a bore 38 in a wall of the sample holder

5   opposite optical fibre 44. Fibre 46 communicates with cavity 42 to receive radiation impinging upon the portion of fibre 46 which communicates with cavity 42. In an alternative embodiment, optical fibers are arranged to permit measurement of reflected light in a sample.

Radiation is channelled through optical fibre 44 to the plasma

10   specimen in a section of tubing 32, and the radiation transmitted through the tubing and markings on the tubing, and plasma specimen, is received by fibre 46, which returns collected radiation to spectrophotometer 14. In a preferred embodiment, fibres 44 and 66 are both 0.4 millimeter diameter, and referring also to Figures 1 and 4, fibre 48 is 1.6 millimeters, and fibre 46 is 0.5

15   millimeter. The reference fibres 66 and 48, which are of different diameters, are coupled together by a coupler 52. Although specific sizes of these fibres have been identified it is understood by those skilled in the art that other fibre sizes could be employed.

Referring to Figure 1, the apparatus 10 includes a spectrophotometer

20   14, a central processing unit 16, a power supply 18, and a lamp assembly module 20.

Referring to Figure 4, the lamp assembly module 20 employs a light source 62. Preferably the light source is a quartz-tungsten-halogen 10 watt lamp, but other wattage lamps can be employed. The input power supply is

25   alternating current, but the output to the light source is a stabilized direct current. Attached to the lamp is a photodetector 80, which monitors lamp output. Spectral output from light source 62 is broad band covering visible and NIR regions. Although the NIR region of the electromagnetic spectrum is generally considered to be the interval extending from 650 nm through to

30   2,700 nm, the nominal wavelength range of a preferred embodiment is from 475 nm to 1,075 nm, which is referred to herein as the "near infrared and adjacent visible region". The beam of radiation from light source 62 is

a choice of integration time for the sample beam. Because the sample holder is not light-tight, sample and reference dark scans be subtracted from sample and reference light scans respectively; sample and reference dark scans are performed at the same integration times used for the respective light scans. In a preferred embodiment, the reference scan is performed at 13 milliseconds, and the sample scan is performed at 20 milliseconds; the maximum ADC value obtained at 20 milliseconds for a particular sample, is used to determine a new integration time up to 2600 milliseconds, such that saturation of the detector at any pixel does not occur. The maximum time allowed for any sample will depend on required speed of sample screening. Also, multiple scans can be averaged to minimize noise, but for the sake of speed in a preferred embodiment single scans are used.

When in use, each pixel or wavelength portion is measured approximately simultaneously during a particular scan. Optical radiation falling on each sensor element is integrated for a specified time and individual pixels or wavelengths are sampled sequentially by a 16 bit analog-to-digital convertor or ADC.

Although the present embodiment details use of a PDA, any alternative means which achieves the same result is within the scope of the present invention. For example a filter-wheel system may be used. In carrying out measurements each analyte uses from one to four wavelengths or pixels. Given that the first derivative of absorbance with respect to measurements with the PDA is the difference between the absorbance at two adjacent pixels, the first derivative of absorbance at one wavelength with a filter-wheel system will require absorbance measured with two different narrow band-pass filters. It will be readily understood by those skilled in the art that the filters do not need to be assembled on a rotating wheel, but that any structure which achieves the result of a narrow band-pass filtration of absorbed radiation is within the scope of the present invention.

Transmission is preferred over reflectance although either may be used. Variations in apparent absorbance due to markings on tubing can be accounted for by using the first derivative of apparent absorbance. The term

converter or ADC. The digital information from the converter is interpreted for data analysis by a microprocessor 16 which is in turn connected via an RS232 connector to a computer 84. The results of the data analysis can be displayed on the computer 84, or on a printer (not shown in Figure 1) connected to 84. A user can control the device through the computer 84, to specify a particular interferent to be analyzed and to determine the number and timing of measurements.

Although a rapid pre-screening device could take as much time as one to two minutes per sample measurement and still be considered rapid in this field of art, the present invention allows for rapid pre-screening of samples by taking successive sample measurements at intervals of 5 seconds for 4 interferents, (not including MB which will be measured after the MB is added to the plasma). After sample holder 22 is opened, the sample is placed according to a controlling process and a sensor in the sample holder activates the movable half of the holder to close when a sample is in place. Spectral data is collected after the holder is closed. Thereafter the sample is removed and another sample is picked up by the robotic arm and placed into the sample holder to allow for another measurement. This set of operations takes approximately 5 seconds.

The integration time for the sample beam is low for clear sample since there is less scattered light and therefore more light is transmitted to detector 78. When light is sufficiently scattered by, for example a turbid sample, spectrophotometer 14 automatically switches to a higher integration time. The higher integration time chosen will be within a pre-selected range, such that the detector's response is optimal. This feature will allow all samples, from the clearest to the most turbid, to be efficiently screened without exceeding the linear response range of the detector.

It is understood that this invention can be used with all varieties of tubing material as typically encountered in the blood bag industry.

As with any quantitative method, calibration of the spectrophotometer is required. However the method for NIR calibration is much more complex than most which can be calibrated with a minimum of a single standard

17

and such calculations are within the scope of this invention. However, each step of taking differences to calculate those derivatives is more time consuming and introduces more noise.

In practice, an optimal combination of first derivatives of at least two portions of an absorbance spectrum generated from a scan of a plasma specimen containing a particular interferent, is used to calculate interferent concentration. The precise approach used depends on the interferent being measured.

In respect of Hb results may be obtained by calculating the first derivative of absorbance measurements at wavelengths of approximately 591nm and 653nm. In respect of turbidity results may be obtained by calculating the first derivative of absorbance measurements at wavelengths of approximately 988nm and 1038nm, or for an alternative algorithm, 874nm. In respect of bile pigments results may be obtained by calculating the first derivative of absorbance measurements at wavelengths of approximately 649nm 731nm and 907nm for BV, and 504nm, 518nm and 577nm for BR. In respect of MB results may be obtained by calculating the first derivative of absorbance measurements at wavelengths of approximately 677nm and 953nm.

Since turbidity or lipemia is mainly due to chylomicron particles, turbidity may be simulated by adding IL to clear plasma; IL is an emulsion of fat particles similar to naturally-occurring chylomicrons.

Calibration equations outlined below cover a broad range of variability anticipated for the interferents. According to the present invention, if low-end accuracy becomes a concern, separate calibrations can be developed: one for the high end, and a second, if the result predicted by the previous calibration is less than a predetermined level.

To calibrate spectrophotometer for use in a preferred embodiment of the present invention, for haemoglobin, IL and BV, plasma specimens with normal appearance were spiked with 0 to 6 g/L Hb, 0 to 6.5 g/L IL, and 0 to 4.5 mg/dL BV. No significant intercorrelation among the analytes was allowed. The specimens were run once immediately after preparation, and

Figure 8 is a graphic representation of the results of a linear regression fit of the data generated from the BV calibration. The algorithm which was developed for BV based on this data is as follows:

mg/dL BV = -45.40(649nm) + 323.15(731nm) - 493.79(907nm)

5   - 1.14

where (Xnm) is the first derivative of the absorbance measurement at the wavelength specified.

In order to calibrate the spectrophotometer for BR, plasma specimens with normal appearance were spiked with 0 to 42 mg/dL Ditaurobilirubin (a

10   synthetic conjugated bilirubin used to calibrate chemistry analyzers) 0 to 3 g/L Hb, 0 to 3 g/L IL, and 0 to 4 mg/dL BV. No significant intercorrelation among the analytes was allowed. The specimens were run once, immediately after preparation, and then repeated using different segments of PVC tubing with random location of white markings on the surface of the

15   tubing. Hb was prepared by replacing normal plasma (by appearance) with water and lysing erythrocytes through three freeze-thaw cycles. Hb content of the supernatant of the lysate was measured on an Abbott Cell Dyn.™ The spectra were stored on diskettes. The analyses on sample sets were performed by a statistical computer program and algorithms developed for

20   BR. Independent sample sets were set aside for validation (referred to in the graphical representations as prediction) of the calibration equations.

Figure 9 is a graphic representation of the results of a linear regression fit of the data generated from the BR calibration. The algorithm which was developed for BR based on this data is as follows:

25   mg/dL BR = -43.03(504nm) + 252.11(518nm) + 240.03(577nm) - 2.89

where (Ynm) is the first derivative of the absorbance measurement at the wavelength specified.

To calibrate the spectrophotometer for methylene blue, plasma specimens with normal appearance (by appearance) were spiked with 0 to

30   860 µg/dL MB. MB is only added to plasma with normal appearance, therefore calibration for MB does not require the presence of the other interferents. The specimens were run once, immediately after preparation,

WE CLAIM:

1.   An apparatus for determining plasma integrity where said plasma is contained in two bags said apparatus comprising tubing which connects said bags, a receptacle for receiving said tubing a lamp to irradiate plasma in said tubing, and a spectrophotometer to measure radiation from said plasma in said tubing, said measured radiation being correlated with a concentration of at least one known substance in said plasma.

2.   The apparatus of claim 1 wherein said tubing is translucent and contains writing on its surface and said radiation is transmitted through said writing, tubing, and said plasma contained in said tubing.

3.   The apparatus of claim 2 wherein said radiation is reflected from a reflective surface placed behind said tubing.

4.   The apparatus of claim 1 wherein said receptacle is a housing comprised of a stationary part which has a cavity for receiving said tubing and a movable part which can close over said tubing such that substantially all movement of optical fibres directing said radiation to and away from said tubing is eliminated during motion of said movable part of said tubing receptacle.

5.   The apparatus of claim 4 wherein light leakage is compensated for by measuring dark current for both sample and reference measurements.

6.   The apparatus of claim 1 wherein said plasma integrity is assessed by measuring:
haemoglobin concentration as an assessment of haemolysis;
bilirubin concentration as an assessment of bilirubinemia;
biliverdin concentration as an assessment of biliverdinemia;

23

means for selecting an appropriate integration time for adequate detector response;

means for correlating a detector response, from said sample path relative to a detector response from said reference path, to a quantity of a known substance in said plasma in said bag.

11.   The apparatus of claim 7 wherein said plasma integrity is assessed by measuring:

haemoglobin concentration as an assessment of haemolysis;

bilirubin concentration as an assessment of bilirubinemia;

biliverdin concentration as an assessment of biliverdinemia;

equivalent intralipid concentration for the assessment of turbidity; and

methylene blue concentration as part of viral inactivation quality assurance system.

12.   The apparatus of claim 7 wherein said receptacle is a housing comprised of a stationary part which has a cavity for receiving a bag and a movable part which can close over said bag, such that substantially all movement of optical fibres directing said radiation to and away from said bag is eliminated during motion of said movable part of said bag receptacle.

13.   The apparatus of claim 12 wherein light leakage is compensated for by measuring dark current for both sample and reference measurements.

14.   A method for determining plasma integrity where said plasma is contained in two bags said method comprising

providing a tubing and connecting said bags with said tubing such that plasma can flow from said bags into said tubing

providing a receptacle for receiving said tubing providing a lamp to irradiate plasma in said tubing, and

placing said bag in a receptacle

providing a receptor having walls contained in said receptacle said receptor shaped so as to accept a corner of said bag;

providing a lamp to irradiate plasma in said bag, and

a spectrophotometer to measure radiation from said plasma in said bag, such receptacle with said receptor configured such that radiation from said lamp is transmitted through said plasma in said bag, and said receptor maintains a constant pathlength across the walls of said receptor for any bag, said measured radiation being correlated with a concentration of at least one known substance in said plasma.

21.   The method of claim 20 wherein said bag is translucent and contains writing on its surfaces, and said radiation is transmitted through said writings, plastic, and said plasma contained in said bag.

22.   The method of claim 21 wherein said radiation is reflected from a reflective surface placed behind said bag.

23.   The method of claim 20 wherein said spectrophotometer comprises: appropriate filters, a grating, a linear photodiode array detector; means for optically connecting said radiation lamp with said detector along a sample path through said receptacle and along a reference path which by-passes said plasma in said bag; means for selectively passing a beam from said sample path and from said reference path to said detector; means for selecting an appropriate integration time for adequate detector response; means for correlating a detector response, from said sample path relative to a detector response from said reference path, to a quantity of a known substance in said plasma in said bag.

24.   The method of claim 20 wherein said plasma integrity is assessed by measuring:

28.   The method of claim 27 wherein said known substance is selected
       from a group comprising haemoglobin, bilirubin, Intralipid, biliverdin,
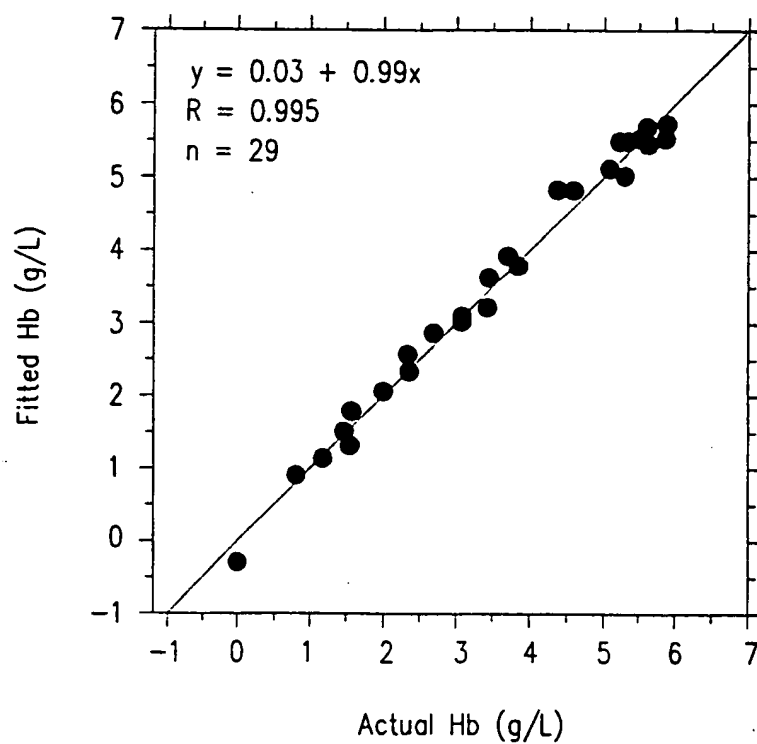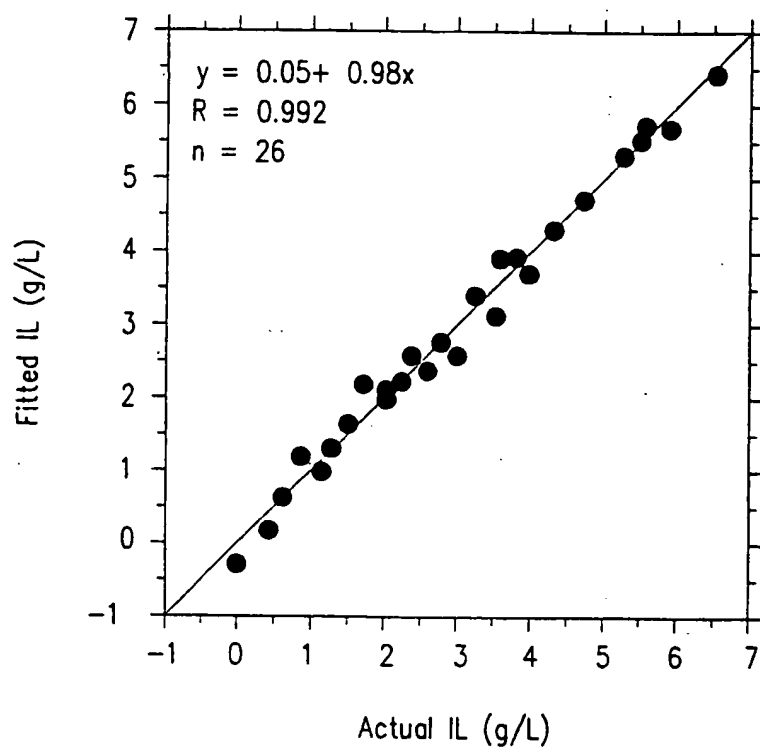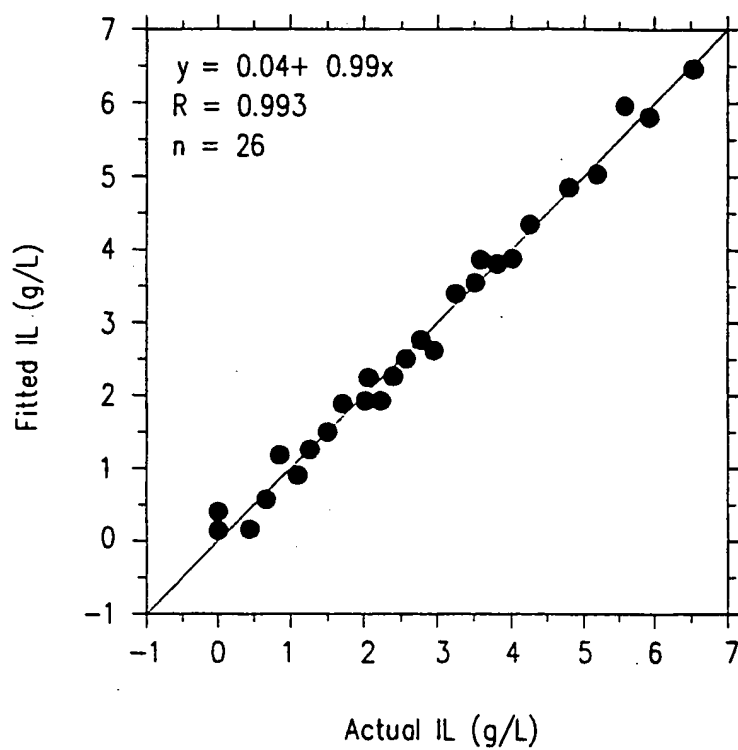   and methylene blue.

FIGURE 1

1/15

FIGURE 2

FIGURE 3

2/15
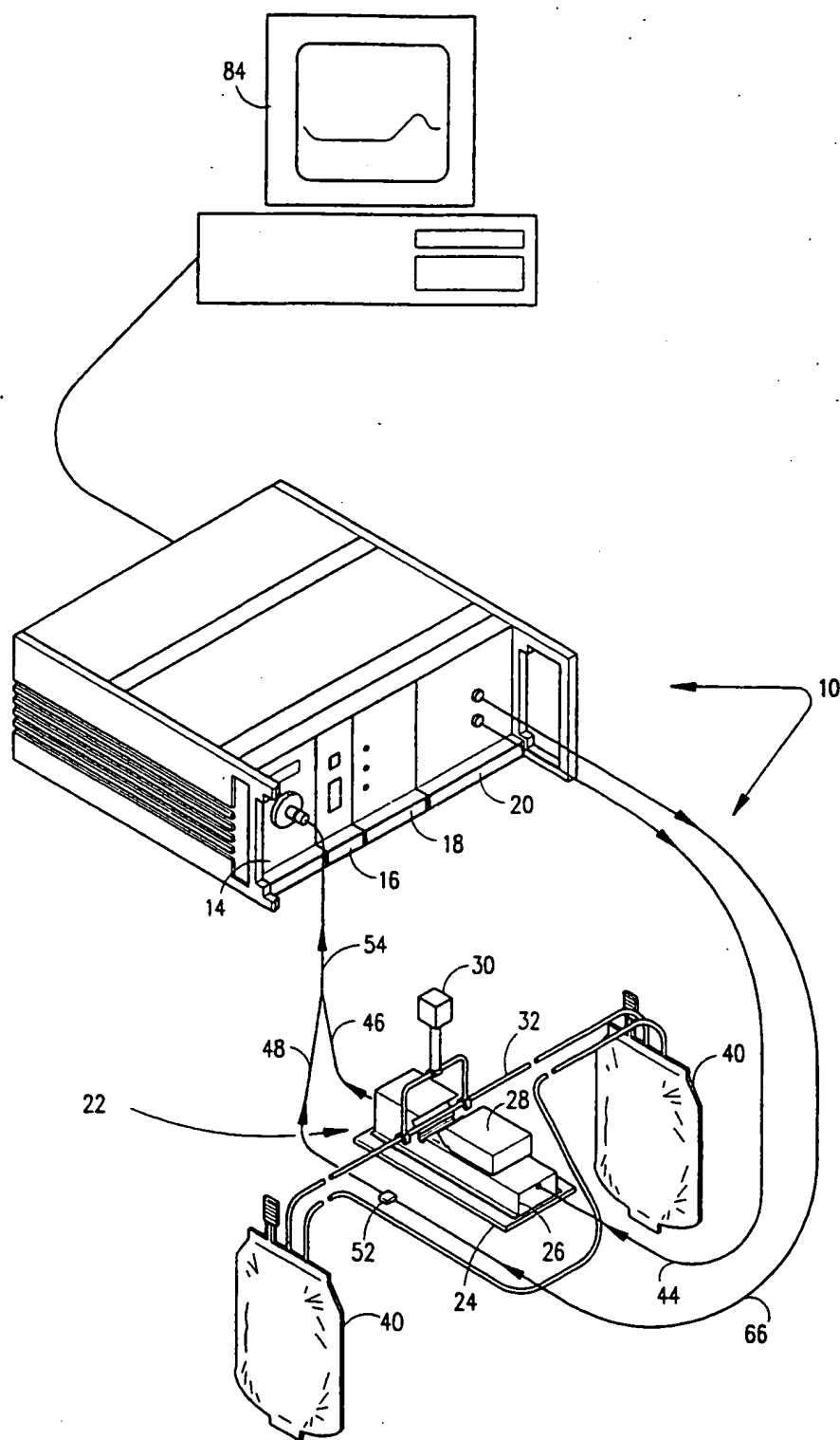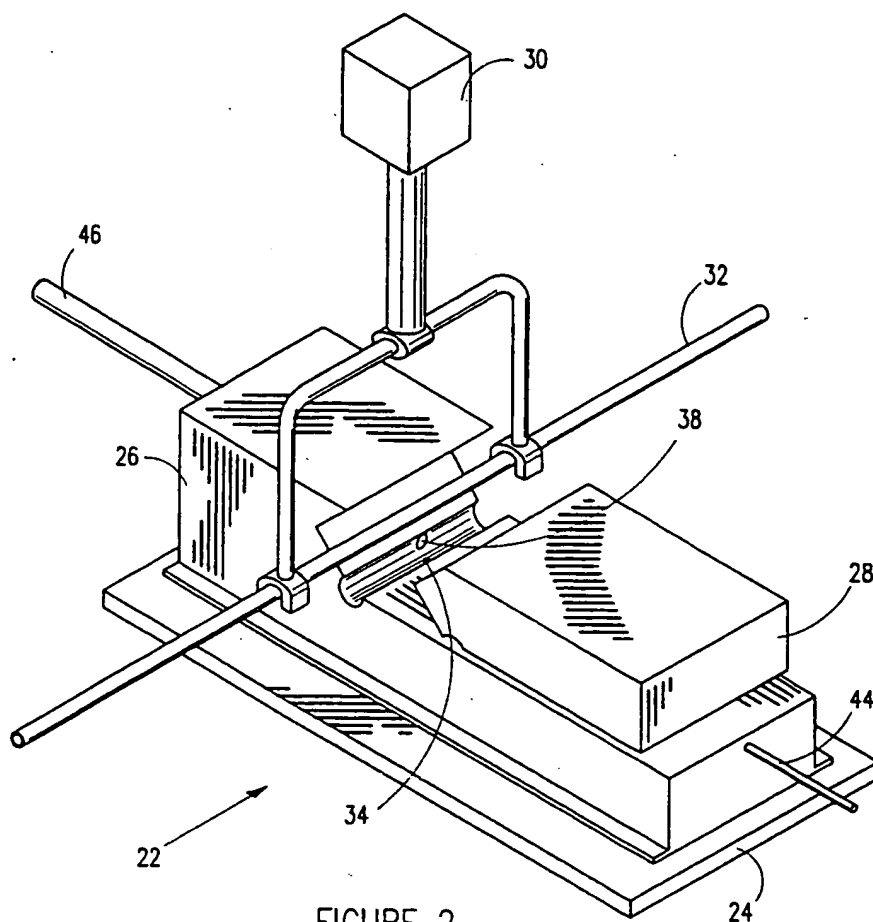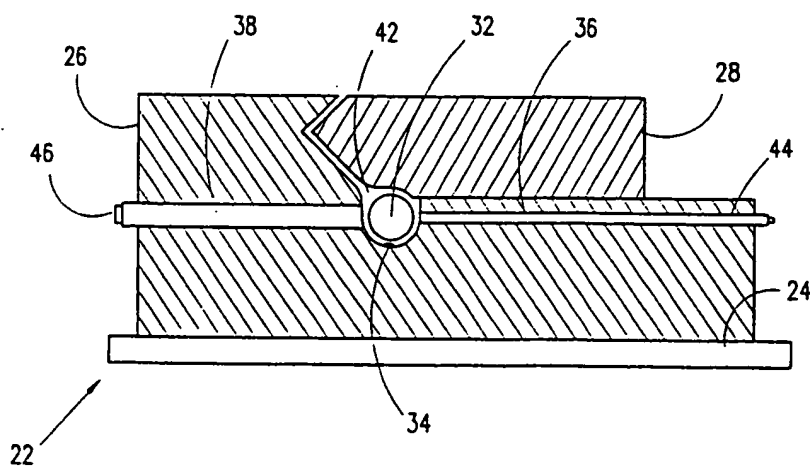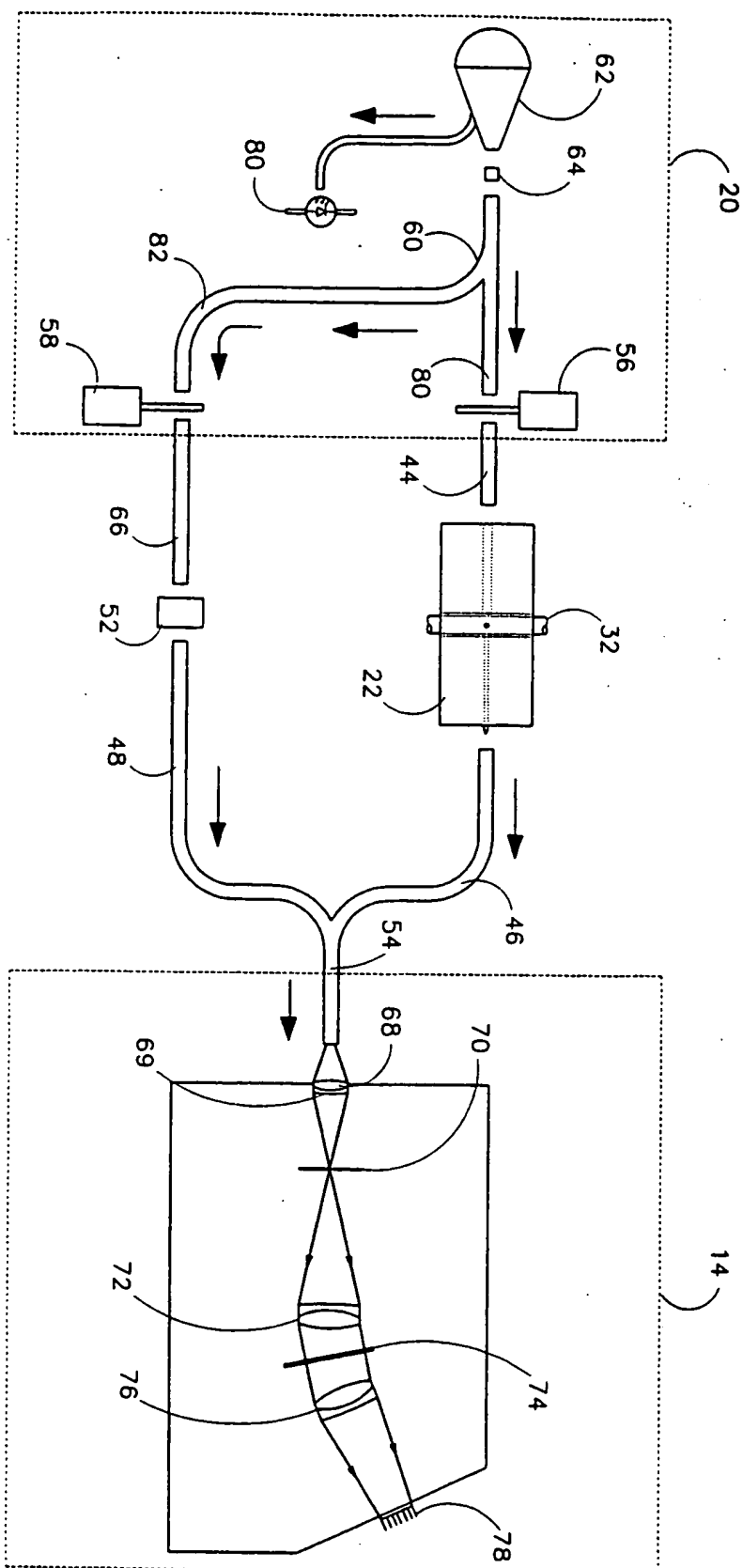
FIGURE 4

Figure 5

Figure 6

5/15

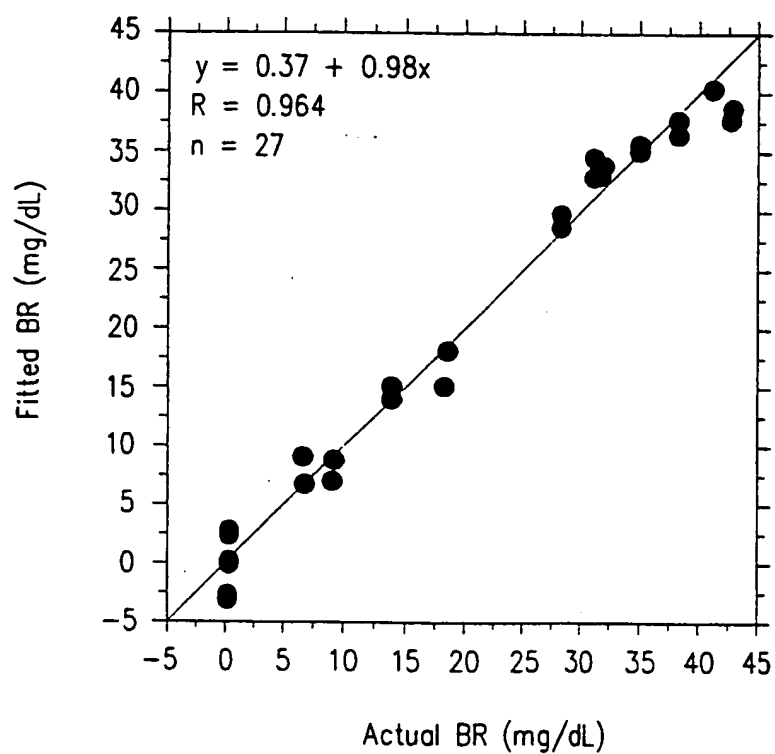Figure 7
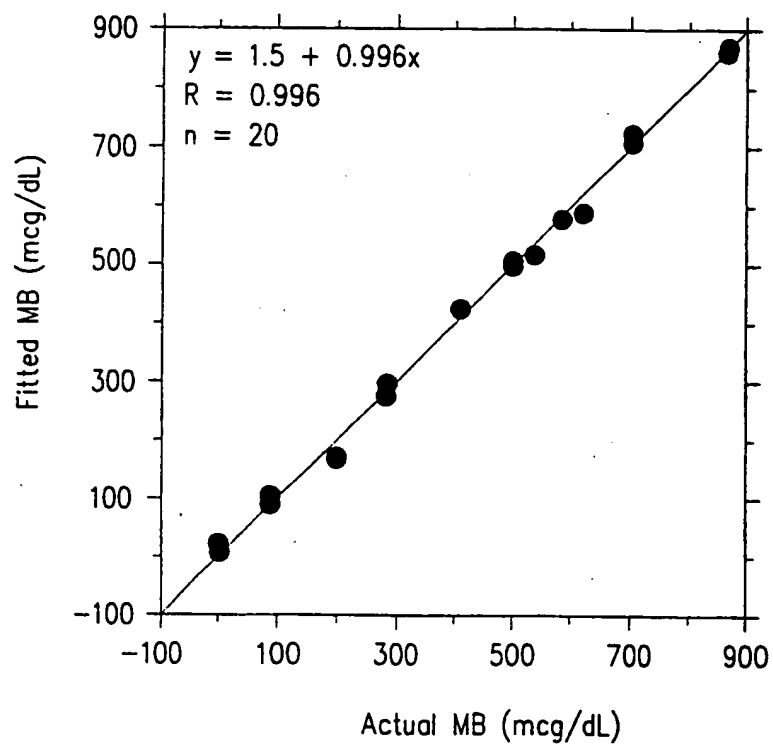
6/15

SUBSTITUTE SHEET (RULE 26)

Figure 8

Figure 9

SUBSTITUTE SHEET (RULE 26)
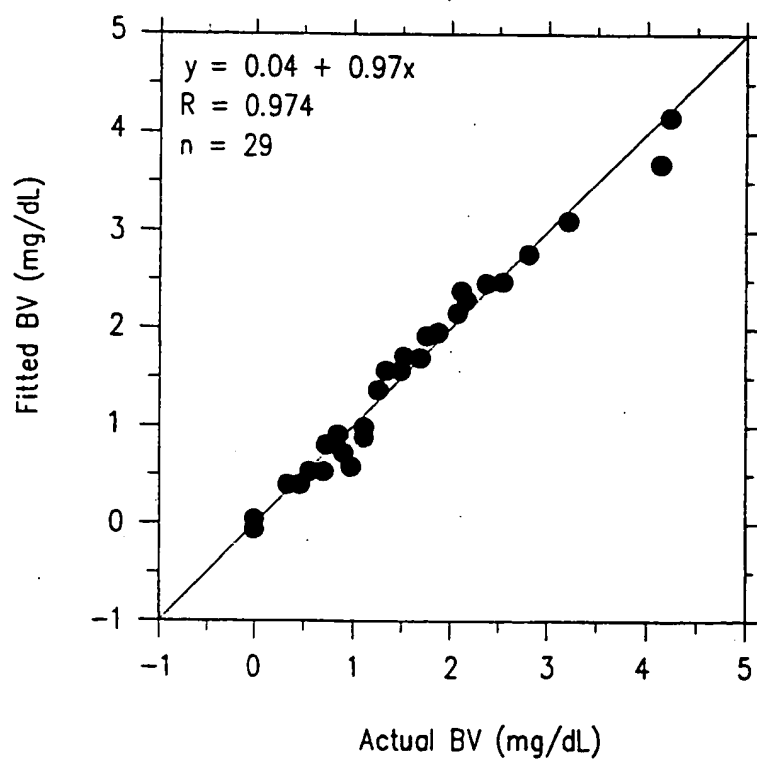
Figure 10

9/15
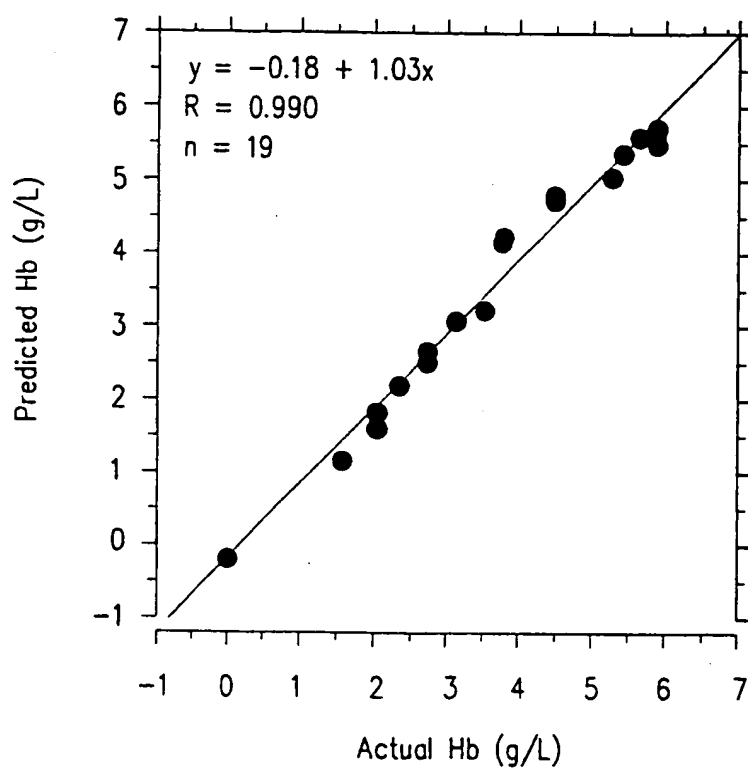
Figure 11

10/15

Figure 12

11/15

$y = 0.03 + 1.01x$
$R = 0.978$
$n = 19$

Predicted IL (g/L)

Actual IL (g/L)
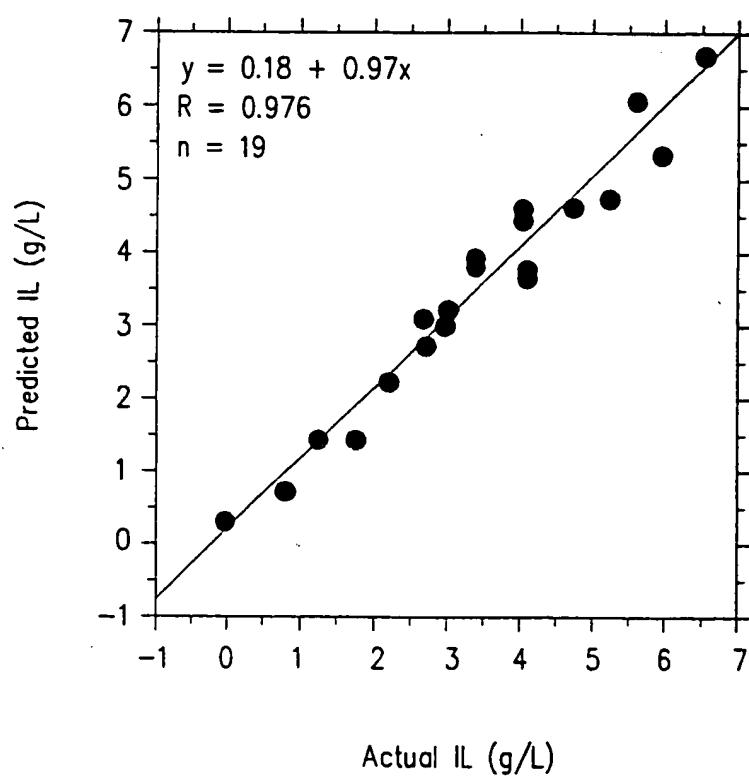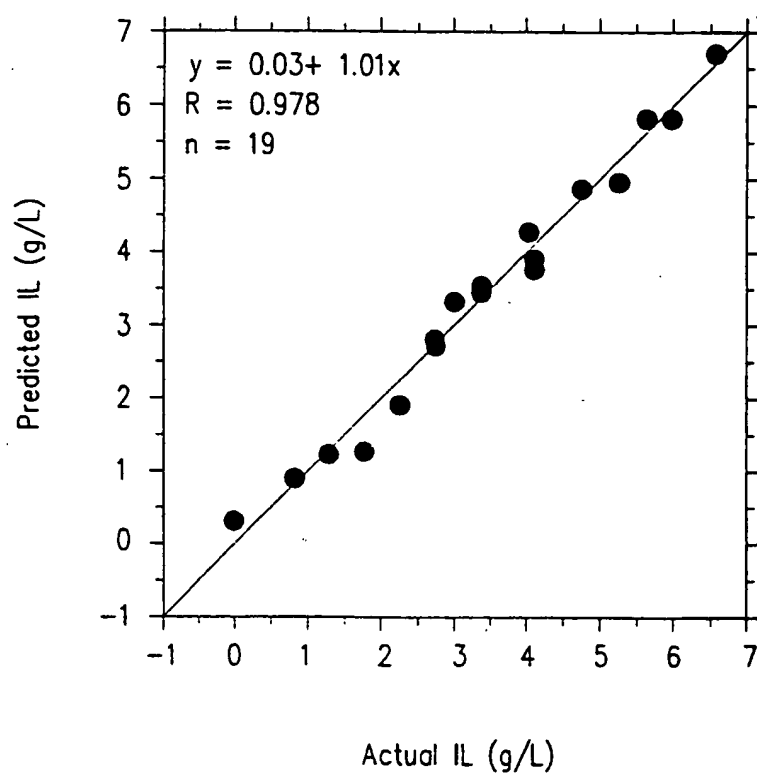
Figure 13

12/15
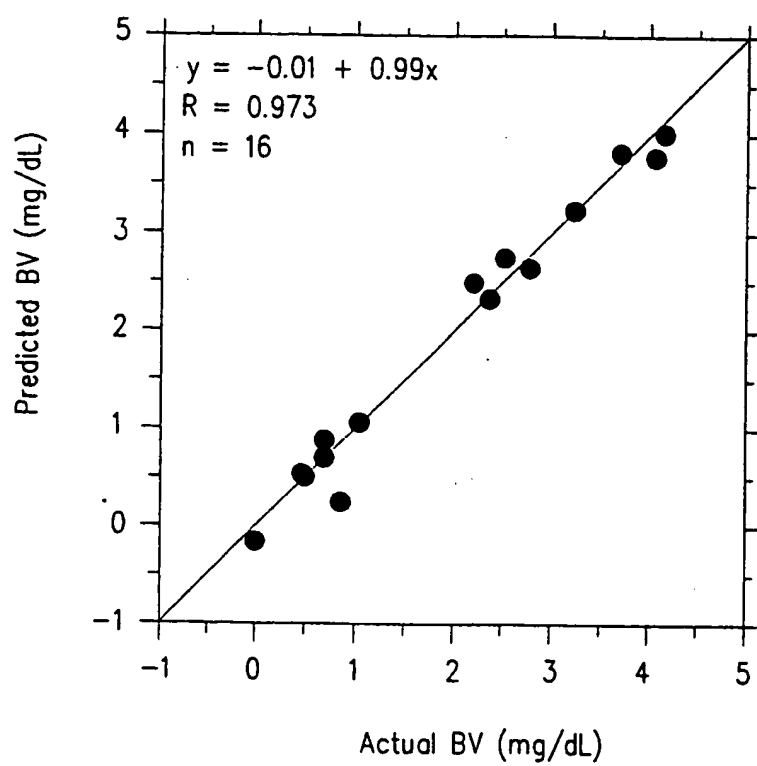
Figure 14

13/15
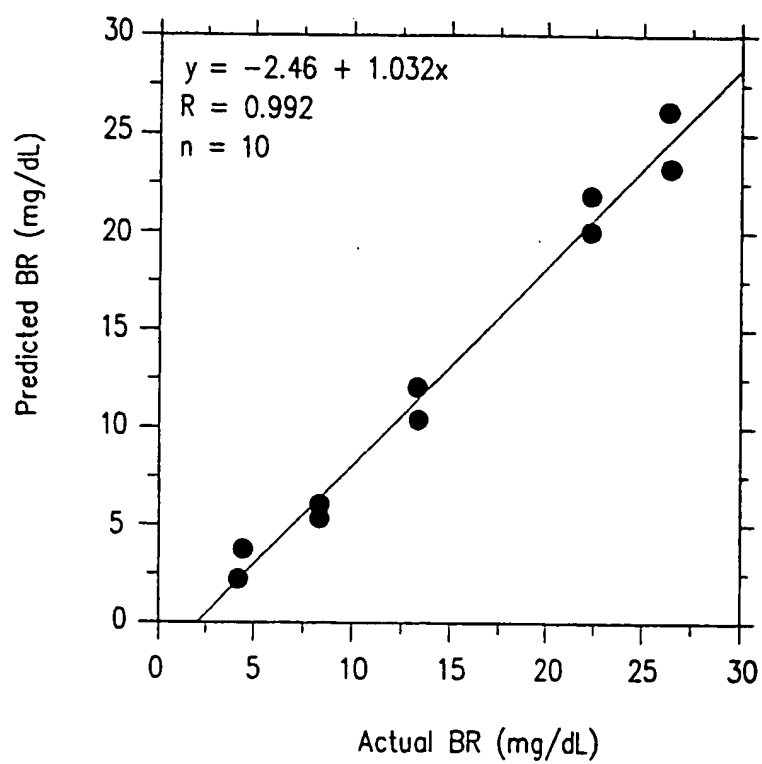
Figure 15

14/15
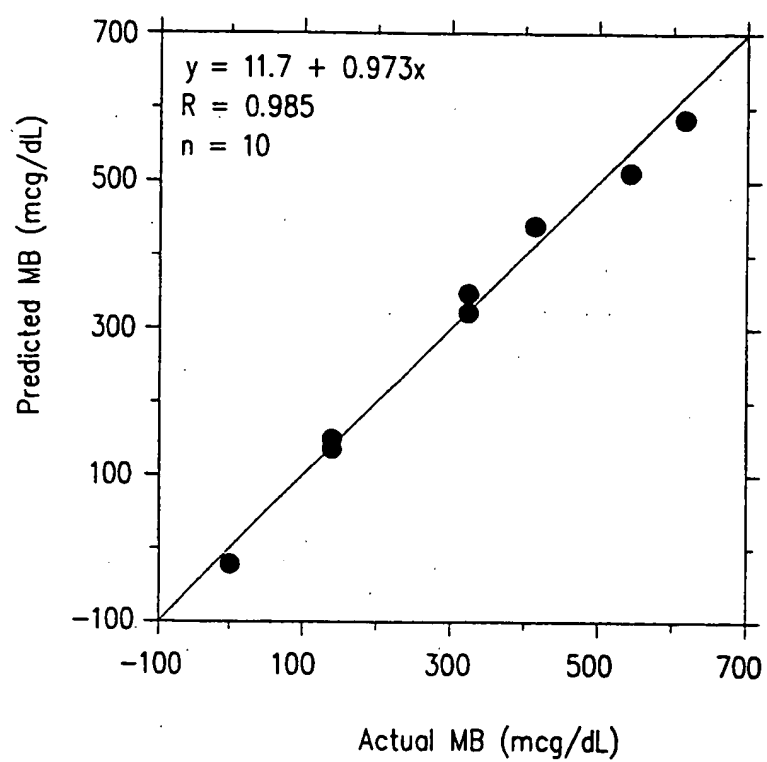
Figure 16

15/15

INTERNATI AL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61J1/00   A61M1/02   G01N21/31

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category [*]	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 195 30 969 A (DEUTSCHES ROTES KREUZ BLUTSPEN) 27 February 1997 see column 3, paragraph 2-3 see column 4, line 9-43; figure 1 ---	1,7,11, 20,27
A	EP 0 706 043 A (OPTICAL SOLUTIONS INC) 10 April 1996 see page 3, line 1-35; figure 1 see page 4, line 20 - page 5, line 7 ---	1,7,11, 20,27
A	US 4 522 494 A (BONNER ROBERT F) 11 June 1985 see column 1, line 5-10; figure 2 see column 3, line 30 - column 4, line 33; figure 2 ---	1,7,11, 20,27

-/--

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

[*] Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 1998	27/07/1998

| Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016 | Authorized officer

 Zinngrebe, U |

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
DE 19530969	A	27-02-1997	WO	9707836 A	06-03-1997
			EP	0846006 A	10-06-1998
EP 0706043	A	10-04-1996	US	5510621 A	23-04-1996
			AU	3304095 A	18-04-1996
			CA	2159235 A	04-04-1996
			JP	8210973 A	20-08-1996
			US	5750998 A	12-05-1998
US 4522494	A	11-06-1985	NONE		
US 4675019	A	23-06-1987	EP	0198869 A	29-10-1986
			WO	8602545 A	09-05-1986
			JP	62500567 T	12-03-1987
			US	4758083 A	19-07-1988